TRACE METALS ASSOCIATED WITH SESTON COLLECTED FROM SPENCER GULF, SOUTH AUSTRALIA, NEAR A LEAD SMELTER: SPATIAL DISTRIBUTION, TEMPORAL VARIABILITY AND UPTAKE BY BIVALVES

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Summary

Ross, K. E., Bidwell, J. R., Williams, M. & Boland, J. (2002) Trace metals associated with seston collected from Spencer Gulf, South Australia, near a lead smelter: spatial distribution, temporal variability and uptake by bivalves. *Trans. R. Soc. S. Aust.* **127**(1), 33-45, 31 May, 2003.

Metal input from point-source discharges results in concentrations in the receiving areas that vary in both space and time. In order to assess the spatial distribution and temporal variability of metals in the Upper Spencer Gulf as a result of metal input from a lead smelter, seston was collected over six week periods at eight sites around the gulf. A total of 23 collections from the eight seston sampling sites was made. Levels of metals in the seston were elevated at the four sites closest to First Creek (the smelter effluent discharge creek). Seston collected from site W6 (the site closest to the smelter) contained the highest levels of all measured metals. Levels of Pb in W6 seston ranged from 973 μg/g to 3060 μg/g, while levels of Zn, Cd, Cu and Mn at this site ranged from 2630 - 9530 μg/g, 41.2 - 356 μg/g, 56.1 - 211 μg/g and 241 - 921 μg/g, respectively. A strong seasonal influence in seston metal levels was found at all sites except W6. The extent of this seasonal influence was evaluated using time series analyses and the influence incorporated to determine overall trends. Seasonal variation accounts for one third to half the variation in deposition annually. Most sites exhibited higher metal levels in winter, corresponding with the decreased seston deposition in that season, and conversely, increased metal concentrations in summer, corresponding with higher deposition rates. There was no significant change over time in deposition rates at any of the sites except W6, for which there was a decrease in total deposition over the monitoring period. Most sites showed statistically significant changes in seston metal levels over the three years of the study. The site closest to the effluent outfall (W6) was the only site that showed a significant increase in metal concentrations over the monitoring period. This study indicates that frequent, regular monitoring of seston metals allows variation arising from seasonal differences to be taken into account, allowing temporal changes to be elucidated. To evaluate bioavailability of metals associated with seston in the Upper Spencer Gulf (for which there were large quantities of metal data), experiments were conducted using scallops (*Pecten alba* Tate) (summer) and scallops and mussels (*Mytilus edulis* Linnaeus) (autumn exposure). Mussels were also placed at the seston sampling site closest to the effluent outfall and removed at intervals over the six week sampling period in autumn to determine the rate of metal uptake. Tissues were analysed for levels of Pb, Cd, Cu, Zn and Mn, and the levels at each site compared with that in the seston. A strong relationship was found between the levels of Pb and Zn in seston and scallop Pb and Zn uptake, whereas Cu and Cd levels in seston showed no relationship with those in scallop tissues. Lead and Zn in mussel tissue were related to Pb and Zn in seston, and there were also weaker relationships between the levels of Cd and Cu in the mussel tissue and seston, although relationships with these metals were not detected in the scallops. The temporal accumulation study indicated that mussels were regulating some metals, with Zn and Mn levels initially increasing, then levelling off. Cadmium levels were high in the scallops collected from the reference site, and showed no significant increase on retrieval after six weeks near the smelter. Cadmium in the mussels increased significantly over the six week exposure period. Copper levels were also high in the scallops from Hardwicke Bay, and decreased significantly after six weeks in summer. Copper levels remained static for both mussels and scallops in autumn. Neither Cd nor Cu showed a relationship between the levels in seston and scallops in either exposure.

During four seston collections, the total organic carbon (TOC) content and the particle size distribution of seston collected from each of the sites was determined. Surprisingly, no relationships were found between either TOC content or particle size of seston and metal concentrations in the bivalves, suggesting that the major influence in tissue metal level is the total concentration of the metals in the seston. These results are confirmed (particularly in the case of Pb and Zn) by the relationships between seston metals and bivalve tissue levels.

Key Words: Trace metals, seston, Spencer Gulf, scallop, mussel, *Pecten alba*, *Mytilus edulis*.

Introduction

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Localised trace metal pollution from industrial or municipal wastes has been identified as one of the major issues facing the Australian marine environment (Zann 1995). Since metal levels associated with water, particulate matter and sediment can vary over time, determining the extent of trace metal enrichment requires not only an understanding of the spatial extent of contamination,

but also of the degree of temporal variability associated with metal levels in the various phases. The aim of this study was to determine the spatial distribution and temporal trends in seston metal levels in the Spencer Gulf, an area that receives an effluent with elevated metal concentrations from one of the world's largest lead/zinc smelters. Seston for the purposes of this study is defined as the living or dead, inorganic and organic particulate matter temporally suspended in the water column (i.e.: that proportion that eventually settles into a collection tube). As an extension of the studies of seston metal levels, uptake of metals by organisms feeding on seston was assessed. Molluscs are commonly used biomonitors (Rainbow 1995), because they are filter feeders, tolerate a range of contaminants and are widely distributed (George 1980; Morse *et al.* 1993; Connel *et al.* 1999). Experiments were conducted using scallops (*Pecten alba* Tate) and mussels (*Mytilus edulis* Linnaeus) translocated from reference sites and placed at the seston collection
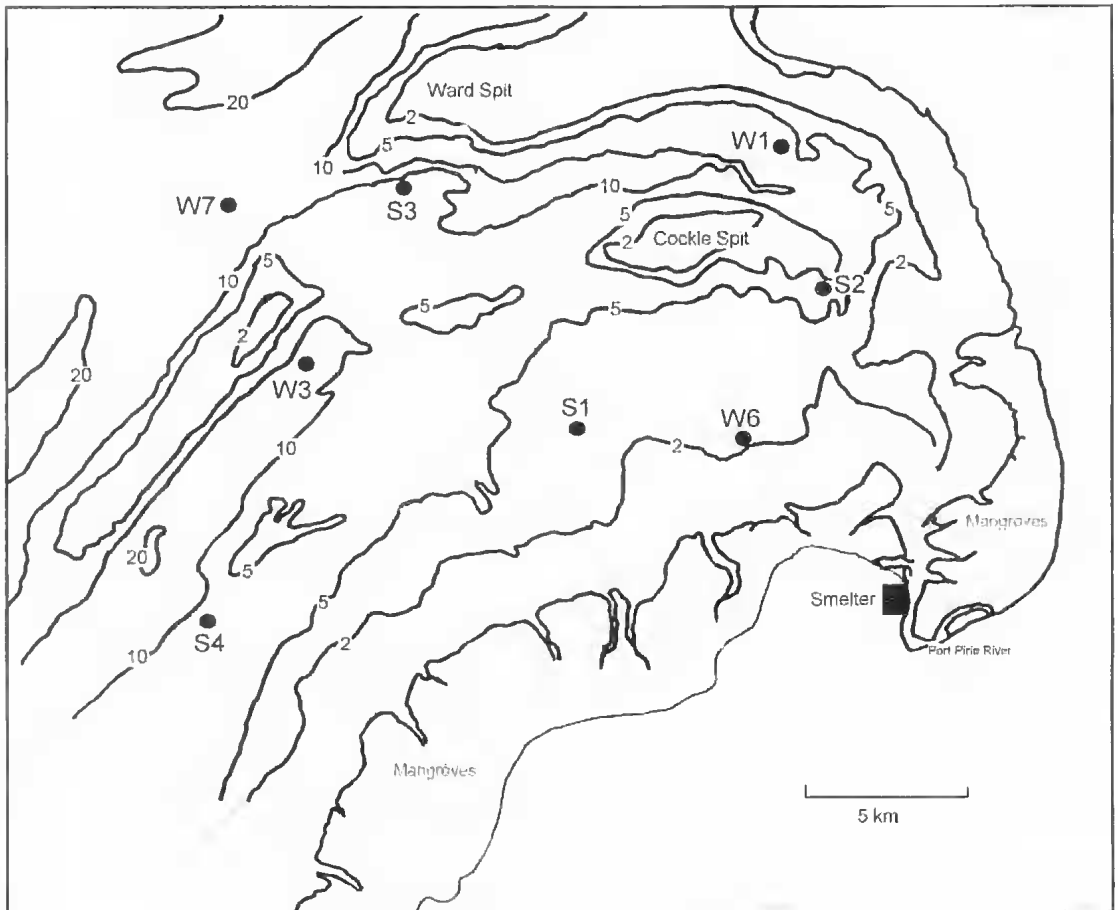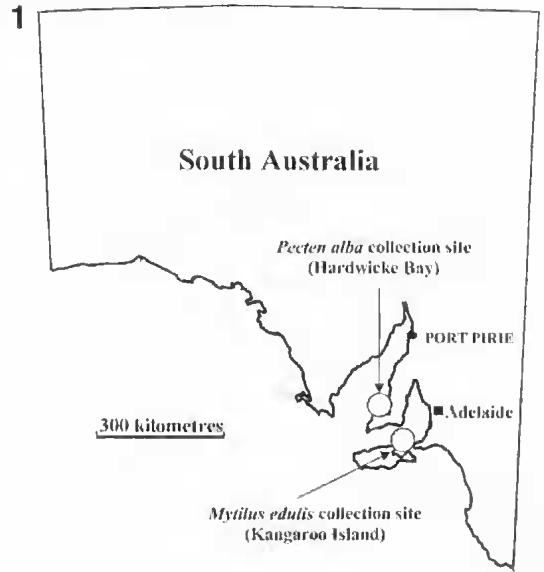

Fig. 1. The study area

sites. *Pecten alba* are indigenous to the area where as *M. edulis* is a cosmopolitan species suitable for drawing between-site comparisons (Rainbow 1995).

The study area

The smelter is located at Port Pirie on the Spencer Gulf of South Australia (Fig. 1) and has been in operation since the 1890s. Processing mainly Pb and Zn, in addition to lesser quantities of other metals and alloys, the facility has been a source of metal input into the gulf for more than one hundred years. The extent and impact of elevated metal levels was assessed through a series of studies conducted in the late 1970s and early 1980s (Ward *et al.* 1982; 1984; Ward & Young 1984; Ward, *et al.* 1984; Ward & Hutchings 1996). In addition to some of the highest ever reported levels of metals in sediments (5000 μg/g Pb, 16,000 μg/g Zn and 260 μg/g Cd), these studies also demonstrated that marine plants and animals (for example: *Posidonia australis, Pinna bicolor, Penaeus latisulcatus, Portunus pelagicus*) near the smelter had accumulated metals in their tissues (Ward *et al.* 1982). Ecological effects included the absence of some species from communities in heavily contaminated sites and the presence of some species that appeared particularly resistant to high metal levels (Ward *et al.* 1982).

Historically, waste metals from the Port Pirie smelter were derived from stack emissions and dust blown from the site, release of liquid effluent and loss of metal ores and concentrates during loading of ships (Ward *et al.* 1982). Most of these sources have been minimised or eliminated as facilities were upgraded and more rigorous environmental policies adopted. However, the wastewater effluent continues to be a significant source of trace metals, notably Pb, Zn, Cd, Cu and Mn (Ward *et al.* 1984). Currently, the average effluent discharge levels are around 80,000 m³/day. The effluent is released into a rapidly flowing small tidal creek (First Creek) that leads to the open waters of the gulf. In their studies of the smelter receiving system, Ward *et al.* (1984) measured trace metal levels in seston. They chose this phase because a large proportion of the organisms within this system are filter feeders (Ward *et al.* 1984). The objective of the study was to again examine metal levels in seston because of the ecological significance of this phase.

Materials and Methods

Sampling

Seston samplers (Fig. 2) were placed at eight sites (S1, S2, S3, S4, W1, W3, W6, W7) (Fig. 1). The W-sites approximate those used in the study by Ward *et al* (1982) The S-sites were chosen to follow prevailing currents and tidal movements within the

TABLE 1. *Site Co-ordinates for Seston sampling sites.*

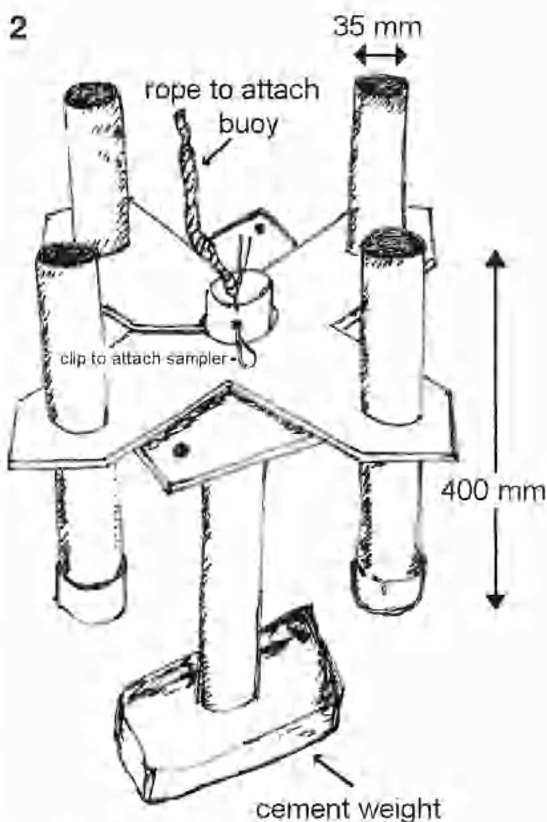| Seston sampling site	Site co-ordinates
S1	33° 07.982′ S 137° 54.298′ E
S2	33° 05.605′ S 137° 58.724′ E
S3	33° 03.914′ S 137° 51.136′ E
S4	33° 10.766′ S 137° 47.682′ E
W1	33° 03.644′ S 137° 58.010′ E
W3	33° 06.691′ S 137° 49.449′ E
W6	33° 08.034′ S 137° 57.728′ E
W7	33° 04.422′ S 137° 48.167′ E

Fig. 2. Seston sampler

area (Ferguson 1983) (Table 1; Fig. 1). The seston samplers were made of PVC tubing (*id* = 35 mm x 400 mm long, total volume = 385 ml), with inserted baffles to prevent re-suspension of collected seston (four tubes per site). The samplers were attached to a concrete block such that the opening of the tubing facing up was approximately one metre above the sediment (Ward *et al.* 1984). Two buoys were attached to the samplers to make the site visible. The collected seston was recovered as close to six week intervals as weather conditions allowed. Samples were retrieved by pulling them from the water in a

manner to minimise disturbance of the collected seston. Each tube was capped and the samplers returned to the laboratory. On retrieval, new tubes were placed in the sampling rack to initiate the next collection. Twenty three collections at the eight seston sampling sites were made between July 1997 and November 2000.

Seston digestion and analysis

Seston collected from each tube was filtered through pre-weighed filters (Whatman® #2 ~ 25 μm followed by 0.45 μm filters Millipore®), the filters and seston dried to a constant weight (~70°C). The first 13 sets of samples were digested with 20 ml 70% HNO₃ (Analar®) and 10 ml 70% HClO₄ (Analar®). Five millilitres 37% HCl (Analar®) was added to the aqueous fraction remaining from the digestion and was made up to 50 ml with Milli RO® water. The other ten sets of samples were digested in H₂O₂ and HNO₃ followed by HCl (the change due to a change in policy of regarding the use of HClO₄ at our research institution) and made to 50 ml with Milli RO®. Filter paper, acid blanks and river sediment reference standard (Standard Reference Material #1645, Washington, DC) controls were used. Samples were analysed for metal concentrations using atomic absorption spectroscopy (Varian®). The difference in results for the two digestion techniques found in the sediment standard were used to 'correct' latter samples for differences in metal recovery arising from the different digestions.

Seston characterisation

During four of the collections in autumn, the total organic carbon (TOC) content and the particle size distribution of seston collected from each of the sites were determined. The particle size fractions above and below 75 μm were determined by wet sieving and subsequent drying at 100°C for 24 h. The fraction below 75 μm was further characterised using a Laser Mastersizer (Malvern Instruments Ltd). All fractions (above 63 μm, 63-4 μm and those below 4μm) are reported as a percent dry weight of the total sediment. TOC was determined using the modified Mebius procedure described by Nelson & Sommers (1982).

Pecten alba and Mytilus edulis studies

Two separate exposure studies were undertaken. In the first (summer exposure: 20/11/98 – 4/1/99), P. alba were the only organisms used. In the second (autumn exposure: 6/4/99 – 18/5/99), scallops and M. edulis were used and additional mussels were placed at the site closest to the effluent outfall and collected at intervals to assess temporal uptake of trace metals. Scallops were collected from

Hardwicke Bay on the Yorke Peninsula of South Australia, and mussels from Kangaroo Island, approximately 15 km from the South Australian mainland. These sites had previously been reported to have low metal concentrations (Zann 1995; Ward et al. 1984). Both species were held in flow through tanks for several days for depuration prior to translocation. Thirty scallops (20 in summer and 10 in autumn) and nine mussels were retained for digestion to establish initial trace metal concentrations (designated as reference concentrations). Mussels (placed first in mesh orange bags) and scallops were placed in plastic cages attached to the seston samplers at each seston sampling site (Fig. 1) and left in place for six weeks. Scallops were placed at all sites in the summer exposure and at sites S2, S3, S4, W1 and W7 in the autumn exposure. Mussels were placed at all sites except W3 in the autumn.

At the end of the field exposure, scallops and mussels were returned to the laboratory and each whole body dried to a constant weight at 70°C prior to digestion in HNO₃ and HClO₄ (Analar®). Tissues were analysed for levels of Pb, Cd, Cu, Zn and Mn using atomic absorption spectroscopy. A biological standard, mussel tissue (GBW 08571) obtained from the National Research Centre for Certified Reference Materials (CRM), (Graham B Jackson Pty Ltd, Victoria, Australia) was digested and analysed in the same manner as the samples.

Temporal uptake

Mussels were placed at W6 (the seston sampling site closest to the effluent outfall) and 5 to 6 individuals removed for analysis on days 3, 7, 14, 28 and 42 over the six week exposure period in autumn to determine the rate of metal uptake. Only M. edulis was used for this study since the location was both shallow (and therefore subject to rapid temperature fluctuations) and had high levels of suspended solids.

Statistical analyses

Initial evaluation of the data sets indicated a seasonal influence on both seston deposition and metal concentrations at most sites. Time series analyses were therefore conducted on each metal and deposition data set by re-ordering the data by time of year, irrespective of year collected. Cosine curves:

$$y = \chi + a \cos(2\pi t, T + b)$$

where: χ = mean metal concentration over the sampling period at each site, a and b are frequency and magnitude variables. $T = 365$ days, and $t = $ day of collection (of a possible 365) were fitted to these re-ordered data sets, which allowed frequency and

Table 2. Seasonal relationships the relationship between season and metal level/deposition (as determined by the coefficient of determination (R² values between empirical data and fitted curve values).

	S1	S2	S3	S4	W1	W3	W6	W7
Pb	0.17	0.34	0.45	0.36	0.56	0.19	0.43	0.11
	-14.13,-3.094	-77.68,-0.394	-33.20,-0.748	-30.67,-0.959	-71.60,-0.533	-20.36,-1.587	-518.19,0.788	-8.93,-1.044
Zn	0.01	0.44	0.60	0.43	0.42	0.30	0.47	0.13
	-32.38,-0.646	-125.62,-0.668	-33.29,-0.777	-61.47,-1.000	-87.50,-0.510	-21.27,-1.540	-1420.26,0.992	-11.07,-1.140
Cd	0.05	0.24	0.24	0.20	0.50	0.15	0.22	0.05
	0.689,-18.44	-2.485,-1.030	-0.531,-0.634	-1.017,-1.070	-1.629,-0.748	-0.703,-3.018	-50.235,1.462	0.443,-18.54
Cu	0.05	0.36	0.55	0.33	0.61	0.15	0.51	0.02
	1.331,0.318	-6.95x(0.015	-2.826,-0.453	-3.251,-0.885	5.857,8.992	-1.655,-1.869	-41.767,1.058	-0.422,-1.192
Mn	0.39	0.25	0.13	0.57	0.28	0.04	0.10	0.04
	-24.775,0.238	-43.174,-0.294	9.952,-1.503	-20.975,-0.670	-30.085,-0.412	5.273,3.057	-79.415,0.178	-3.759,-4.081
deposition	0.33	0.31	0.56	0.40	0.53	0.34	0.095	0.30
	-48.14,-0.429	-40.13,-3.259	116.8,-0.770	-65.48,2.038	-98.83,2.605	-72.80,1.776	-18.24,-0.718	-48.16,-4.161

Upper values indicate the strength of the relationship between empirical data and fitted curve (coefficient of determination). The numbers below indicate the values for a and b in the equation (cosine-curve) $y = \bar{\chi} \pm a\cos(2\pi t\, T - b)$, where $\bar{\chi}$ — mean metal concentration over the sampling period at each site. a and b are indicated on the above table. T = 365 days, and t = day of collection (of a possible 365).

magnitude of change to be characterised. The coefficient of determination (reported as R^2) between the fitted values (as determined by the cosine curve) and the empirical values was determined to assess the strength of seasonal influence. The residuals (the values remaining after seasonal influence was taken into account) were then graphed against time (in chronological order) to deduce any changes in both metal concentrations and deposition over time (Boland 1995). The R^2 values between season and metals level/deposition are indicative of the strength of the seasonal relationship. These residual values are either the result of general (unexplained) variability or can indicate possible temporal trends. Trends in the residual values over time were characterised with regression analyses. All results reported as statistically significant results are at the $\alpha = 0.05$ level. Results where $p < 0.1$ are also included, and are indicated as such. Statistical analyses were performed using Microsoft Excel® spreadsheets.

Seston and bivalve data were compared with regression analyses (least-squares regression with seston metal the independent variable and bivalve metal the dependent variable) using Microsoft Excel spreadsheet software. The relationship was considered significant at $p < 0.05$. The R^2 values (coefficient of determination) indicate what percentage of the variation in the bivalve metals is due to the relationship with the seston metals, and thus a measure of the strength of the relationship. Regression was used since it allowed extreme values that could be skewing the relationship(s) to be removed and the resulting line compared with the initial regression line. TOC and particle size were compared with bivalve metal concentrations using correlation analyses. Scallop trace metal uptake during different seasons and mussel and scallop uptake were compared using correlation analyses. These results were also considered significant when $p < 0.05$, and are reported as the correlation coefficient squared (R^2) (Zar 1984).

Results

Spatial distribution

Levels of metals in the seston were elevated (compared with the other sites) at the four sites closest to First Creek and the shipping channel. Seston collected from site W6 contained the highest levels of all five significant metals. Levels of Pb in W6 seston ranged from 973 µg/g to 3060 µg/g, while levels of Zn, Cd, Cu and Mn at this site ranged from 2630 – 9530 µg/g, 41.2 – 356 µg/g, 56.1 – 211 µg/g and 241 – 921 µg/g, respectively. Metal levels were also elevated in seston from site S2. This site is located approximately 5 km from the mouth of First Creek and on the edge of the shipping channel
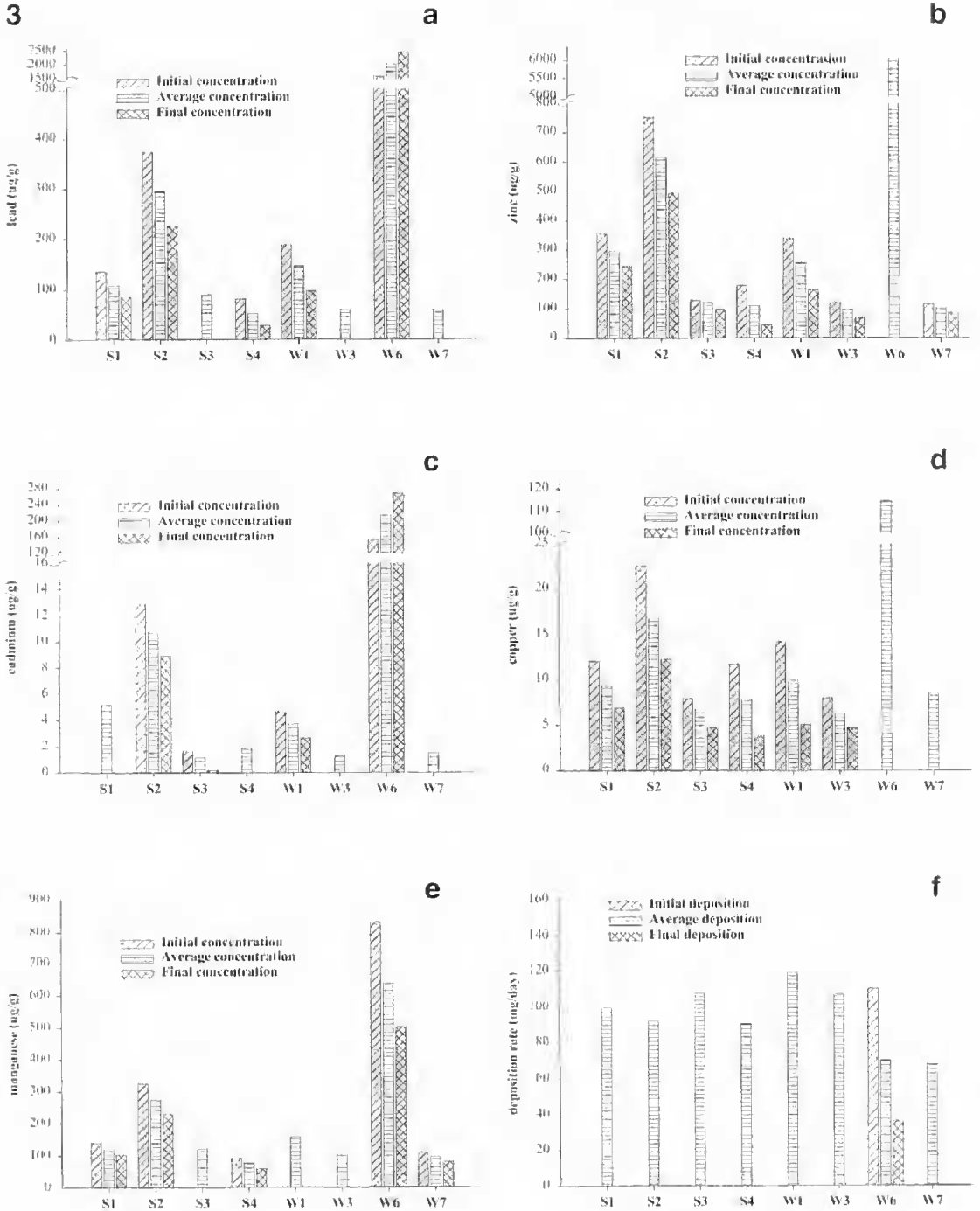
Fig. 3. Levels of lead (a) zinc (b) cadmium (c) copper (d) manganese (e) (mg/g) and deposition rates (f) (mg/day) associated with the seston. Initial and final values indicate the change in seston metal concentration/deposition over the sampling period (1997 - 2000). These are estimated using the residual values (following incorporation of seasonal influence). Initial and final shaded bars indicate p<0.05, unshaded bars p>0.1, no initial and final bars indicates no statistically significant change).

entering the Port River. Levels of Pb in S2 seston ranged from 170 518 μg/g. Sites W1 and S1 had the third highest levels of metals in seston, with levels of Pb ranging from 35.0 222 μg/g. Sites S3, S4, W3 and W7 had lower levels of metals associated with the seston. Mean seston metal concentrations are presented in Fig. 3a-e. Average seston deposition ranged from ~70 mg/day (W6, W7) to 120 mg/day (W1) (Fig. 3f).

Seasonal variation

The influence of season on seston deposition as determined from the model (y = χ − a cos [2πt/T + b]) is presented in Table 2. There were significant (defined as >30% of the variability in deposition explained by seasonal variation) seasonal trends in seston deposition rates at all sites except W6. Season accounted for over half the variation in seston deposition at sites S3 and W1 and approximately one third of the variation in deposition at sites S1, S2, S4 and W7. At all sites except S1, seston deposition was slightly higher in the summer than the winter. Site W6, located closest to the outfall had the weakest relationship between season and seston deposition with an R² value of 0.1. Table 2 also presents the variables associated with the fitted cosine curves (a and b) for each of the metals at each site.

As with deposition, sites W1 and S3 had the strongest relationship between total metal levels and season, with approximately 40% of the variability in metal levels attributed to season at these sites (Table 2). Figure 4 (Zn at S3) is a representative graph illustrating the relationship between season and metal levels. S2 and S4 also had strong seasonal changes in seston metal concentrations. Notably, W6, while showing no seasonal relationship in deposition, was found to have a relationship between total metal levels and season (mean R² = 0.35). W7 had a weak relationship between metals levels and season, despite 30% of the variation in deposition being explained by season. S1 had no relationship between metal levels and season, except for Mn, and W3 had a relatively strong relationship between Zn and season, and weaker relationships between Cd and Cu and season, and none with Mn and season.

All sites exhibiting seasonal variation in seston metal concentrations except S1 had higher metal levels in winter corresponding with the decreased seston deposition in winter, and conversely, increased metal concentrations in summer, corresponding with higher deposition rates.

Temporal trends

There was no significant change over time in deposition rates at any of the sites except W6, for which a decrease in total deposition was observed over the monitoring period (Fig. 3f).

Lead concentrations in the seston decreased significantly at four sites over the course of the monitoring study (S2, S4, W1, S1 – although at site S1 p was <0.1), but increased significantly at W6, with ~30% of the variability at these four sites explained by temporal changes (Fig. 3a). Zn levels decreased significantly at six sites (S1, S2, S3, S4, W1 and W3), and remained unchanged at W6 (Fig. 3b). There was a less significant decrease in Zn seston concentrations at W7 (p<0.1). Figure 5 present examples of strong (S2: R² = 0.56) and poor (W6: R² = 0.01) relationships between change in seston metal levels and time. Cd levels decreased at S3 and W1, and increased significantly at W6 (Fig. 3c). S2 also had a decrease in Cd concentrations, although at a less significant level (p = 0.1). Copper concentrations decreased significantly at all sites (although S1 at p = 0.1), except W6 and W7, with no change (Fig. 3d). Mn concentrations decreased at S1, S2 and S4, and increased at W6 (Fig. 3e).

Those sites showing the most significant changes in seston metal levels were S4 and W1 (decrease in total metals), followed by S2 (decrease), S3, S1, W3, W7. The site closest to the effluent outfall (W6) was the only site that showed a significant increase in metal concentrations over the monitoring period.

Standard reference material

All standard reference material (SRM) Zn, Cu and Mn recovery values fell within the confidence limits of recovery values published by the supplier (1720±169, 109±19, 785±97 μg/g, respectively). Additionally, there was no significant difference between the 2 digestion methods for these three metals. Lead digested with HClO₄/HNO₃ fell within the SRM supplier values (714±28 μg/g), although Pb recovery was lower using the H₂O₂/HNO₃ digestion (by 11%). Cd recovery was lower than the SRM values for both digestion methods, with a difference of 15% when the change was made to H₂O₂/HNO₃. Therefore Pb and Cd values were 'corrected' by multiplying the empirical values by 1.11 and 1.15 (Pb and Cd, respectively). Given the difference in binding constants between Pb and Cd (Balistrieri & Murray 1984), it is not clear why these results were obtained. Nonetheless, the results were consistent and reproducible and were therefore used to allow comparisons between the two digestion methods to be made.

Bivalves

The scallops all died at W6 in the summer and were therefore not placed at this site in autumn. At all other sites there were few or no deaths of either scallops or mussels during the six-week exposure period.

TABLE 3. R^2 values for the regressions of scallop and metal seston levels.

	Pb	Zn	Cd	Cu	Mn
Scallops (summer)	*0.87*	*0.88*	0.15	0.23	*0.74*
Scallops (autumn)	*0.90*	*0.73*	0.04	0.001	0.002
Mussels	*0.99*	*0.92*	*0.62*	0.44	0.001

Numbers in italics indicate a significant relationship ($p<0.05$), underlined numbers indicate no change in (significant) slopes when regression analysis performed on the data points minus S2 and W6.
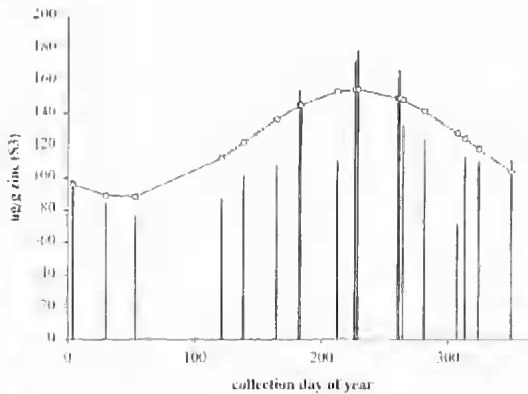
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Fig. 4. Example illustrating the relationship between empirical seston metal concentration and the fitted cosine curve (graph presents zinc at site S3)

Relationships between seston metal and bivalve metal

Table 3 presents a summary of the relationships between the bivalve and seston metal concentrations. There were significant relationships between seston and bivalve Pb and Zn concentrations in both exposures ($p<0.05$). There were also significant relationships between Cd in seston and mussel tissue and between seston Mn and scallop Mn (in summer only). Seston metal levels at site S2 in the scallop study and sites W6 and S2 in the mussel study were significantly elevated compared with other sites. In order to determine the effect these points had on the regression curve, the analysis was also performed with the points removed. The slope of the second regression curve was then compared with that generated with all the data points. Representative graphs illustrating the effect of removing extreme values are presented in Fig. 5.

The slopes of the relationship between Pb levels in scallops and Pb levels in seston were not significantly altered after removing the S2 data point (Table 3). In the mussel exposures, there was no significant change in the slope when the W6 point
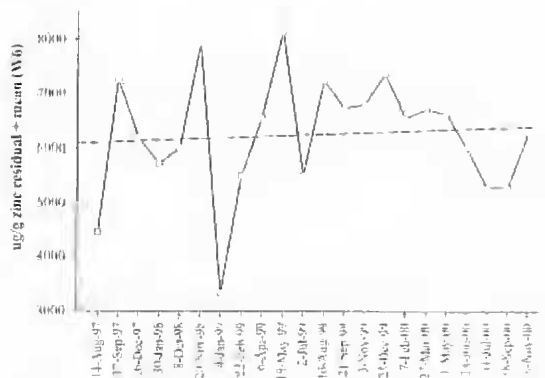
5a
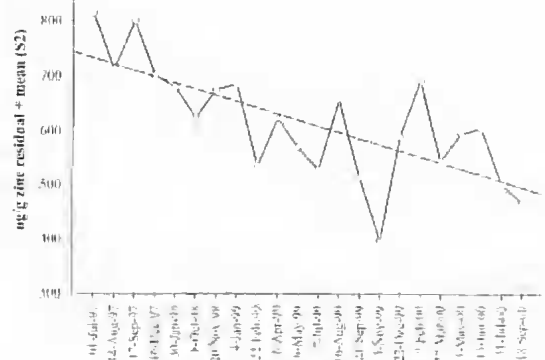
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Fig. 5. Examples illustrating the relationship between seston metal concentration and time after seasonal variation is taken into account. Graphs indicate strong (zinc at site S2) (a) and poor (zinc at site W6) (b) relationships.

was removed, although removing S2 resulted in a 2-fold increase in the slope of the regression curve. There were significant relationships in both Zn comparisons ($R^2 = 0.73$ and 0.88), although when the S2 point was removed from the autumn data, the slopes were no longer comparable. This is possibly the result of fewer remaining data points (4) in the autumn exposure compared with summer (6). The relationship between mussel and seston Zn was significantly altered by removing W6 from the data set ($R^2 = 0.92$ vs. 0.02) although this relationship no longer held when W6 was removed from the data set ($R^2 = 0.02$). This was also true of the relationship between mussel Cd and seston Cd ($R^2 = 0.62$), although no relationship was detected between scallop Cd and seston Cd. There was no relationship between bivalve Cu and seston Cu. There was a significant relationship between Mn levels in scallops exposed in the summer and seston Mn,

TABLE 4. *Summary of R² values found between metal levels in scallops and metal levels in mussels deployed at the seston sampling sites.*

Metal	R² value
Lead	*0.73*
Zinc	*0.55*
Cadmium	0.04
Copper	0.07
Manganese	0.09

Numbers in italics indicate a significant relationship (p<0.05)

although the slope of the regression curve changed significantly when S2 was removed from the data set.

Comparison between P. alba and M. edulis and seasonal comparisons (P. alba)

Lead and Zn were the only metals for which there was a relationship between the two bivalves (Table 4). Levels of Pb in summer exposed scallops were not significantly different from those exposed in the autumn. There was also no significant difference between scallop and mussel Pb concentrations. Scallop Zn was not significantly different between seasons, although scallop Zn concentrations were higher than mussel Zn at all sites. This contrasts with the initial concentrations of Zn in the bivalves, which was slightly higher in the mussel tissues compared with the scallops. Cd levels in scallops exposed in the summer were higher than those exposed in the autumn, and Cd levels in scallops in both seasons were higher than that in mussel tissue. Scallop Mn was significantly higher than mussel Mn at all sites, and also in initial, unexposed bivalves. Table 4 presents a summary of the relationships in metal uptake between mussels and scallops.

TOC and particulate size distribution

There was no relationship between TOC or particle size and uptake of any of the metals examined in either scallops or mussels.

Temporal uptake

Lead and Cd concentrations in mussel tissue increased significantly until day 28, after which the concentrations levelled, indicating mussels had reached steady state. Cd concentrations decreased significantly following initial transfer to the exposure site, while Zn levels increased gradually until day 28. On day 26, concentrations of Zn increased significantly following an accidental release of Zn electrolyte solution from the smelter (2/5/99). The level of Zn in mussel tissue taken on day 46, however, had returned to pre-spill levels indicating some regulation of whole body Zn.

Interestingly, Zn was the only metal for which tissue levels corresponded with the accidental release, although all metal levels were elevated in the effluent (Pasminco Port Pirie, unpub. data). Cu concentrations in the mussels were variable throughout the exposure period, and Mn concentrations decreased following placement at the study site, followed by a significant increase. Mn concentrations did not appear to reach steady state.

Discussion

Spatial distribution of metals

While exhibiting varying binding affinities (Balistrieri & Murray 1984), metals can generally be said to have a preference for the fine particle size fraction of sediments (Lead *et al.* 1999). In this study, seston was not sub-divided into particle size or mass. Morse *et al.* (1993) state that simply reporting average total concentrations can be misleading because of the variation in metal levels associated with different grain sizes. In our study, we attempted to account for some of the influence of particle size by considering seasonal influences on seston deposition and metal levels. Under conditions of low wind and turbulence, suspended particulate matter concentrations are lower and are probably composed largely of finer sediments and conversely, stronger winds resuspend a greater number of larger particles, which are comparatively low in metals (Morse *et al.* 1993). Therefore grain size distribution within seston samples is likely to be the result of physical conditions, which in turn is a result of season in the
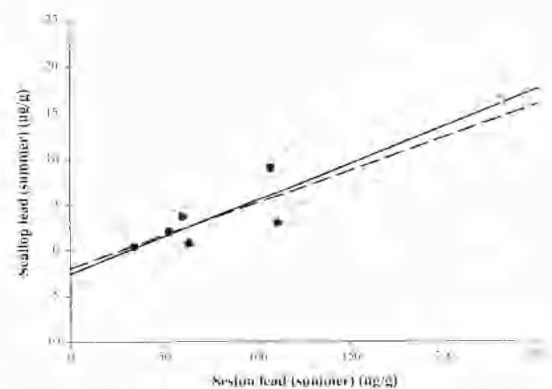

Fig. 6. Graph illustrating a case for which removal of the extreme data has no significant effect (a) and a significant effect (b) on the slope of the line. Unbroken line indicates linear regression line using all data points (+ [grey] 95 % confidence intervals). Broken line indicates linear regression line minus the extreme points [unfilled point] (+ grey dashed 95 % confidence intervals).

Upper Spencer Gulf. By considering seasonal variability, we have a better indication of overall temporal changes in trace metal concentrations.

Seasonal variability does not however account for particle size differences between sites. Normalising for grain size, using metal:Fe ratios or defining the total number of available binding sites are methods that allow between-site comparisons to be made with greater certainty (Morse *et al.* 1993; Turner & Millward 2000; Balistrieri & Murray 1984). This study, however, also assessed the relationship between total seston metal levels and bioavailability. In this study, strong relationships were apparent between seston Pb and Zn levels and Pb and Zn uptake of both scallops and mussels, indicating that seston metals are a good indication of the bioavailable fraction of metals. It is under this assumption that between site comparisons are reported.

Not surprisingly, W6, the site closest to the effluent discharge site had the highest concentrations of the five metals measured. However, seston trace metal concentrations were not a function of distance from the outfall. S1 was the same distance from First Creek as S2, although S2 had higher levels of all five metals. S1 and W1 had comparable levels of metals in seston even though W1 is further away from the outfall than S1 (~ 8 km from W6 compared with 14 km). The concentration gradient for most metals followed the general current flow in the area described by Ferguson (1983). While both S2 and W1 were located on the shipping channel, which could cause resuspension of metal bound sediments, thereby influencing seston metal levels, the comparable deposition rates at S1, S2 and W1 makes that seem unlikely. In a study of metals and particulate matter conducted in the North Sea, Tappin *et al.* (1995) concluded that movement of particulates is generally linked to water circulation and mixing.

The four sites further afield (S3, S4, W3, W7) had significantly lower concentrations of most metals. However, their 'background' status remains unconfirmed (see temporal trends). The levels of Zn and Cd at these four sites were similar to one another and lower than those found at the other four sites (S1, S2, W3, W6), although levels of Pb and Mn at S3 were higher than at the other three sites, as was Cu at W7. This suggests that designating one or more of these sites as representing background levels for all metals is not possible. Our background sites were chosen based on those published by Ward *et al.*

(1982). As noted in the text, however, there were changes in metal concentrations at all sites, suggesting that it is likely that there has been some smelter influence. However, Ward and Correll (1992) argue that remote site, global mean and minimum value methods of estimating background concentrations are less robust than the distance extrapolation method used to choose background sites for this study. Each of the four 'background' sites can be used to determine background concentrations for particular metals, however no one site has no variation in all metals measured.

Seasonal variation

Deposition rates were significantly higher at most sites in the summer months. At that time of year both on and offshore winds are strongest on the Upper Spencer Gulf (Aust. Bureau of Meteorology 2001). Smaal & Haas (1997) also reported significant seasonal variability in suspended particulate matter concentrations in the Netherlands. S1 and W6 did not follow this pattern, probably because of their more protected locations (Fig. 1). S1 deposition was slightly higher in the summer than the winter;the cause for this is not known. W6 showed no relationship between deposition and season, probably resulting from both its proximity to the smelter outfall, with the resulting influence of variability in suspended matter exiting First Creek, and its protected location.

Temporal trends

The lack of change over the three year monitoring period in seston deposition rates at S1, S2, S3, S4, W1, W3, and W7 suggests that only natural influences are playing a role in seston deposition at these sites. Conversely, there was a significant decrease in deposition rates at W6, indicating either an overall decrease in grain size being discharged from the smelter (through increased residence time of the effluent in the ponding process prior to discharge) or, alternatively, that there is continuing 'dilution' of larger, natural particles by the smaller smelter effluent grains. This accumulation of finer grains in areas of decreased flow regimes has been reported elsewhere (Puig *et al.* 1999), commonly occurring when faster flowing creeks meet the open sea.

Generally, metal levels at all sites except the site closest to the smelter outfall (W6) decreased or remained unchanged over the course of the study. This decrease is likely to be the result of the long term (~ 20 year) decrease in the levels of metals being released by the smelter. This conclusion is supported by effluent metal data (Pasminco Port Pirie, unpub. data) and by the decrease in razor fish tissue metal concentrations in the Upper Spencer Gulf reported by Edyvane & Boxall[1]

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[1] EDYVANE , K.S. & BOXALL, V.A. (1996) An investigation of heavy metal contamination of edible marine seafood in the Port Pirie-Telowie Beach region of South Australia. South Australian Research and Development Institute (Aquatic Sciences): Henley Beach, South Australia.

The seston concentration of metals at W6, conversely, either increased significantly or remained unchanged over the monitoring period. There are a number of possible reasons for the different trend in metal concentrations found at W6 compared with the other sites. Firstly, it is possibly the result of the decreased deposition over the same period, due to increased retention time of the effluent prior to release, and correspondingly smaller grain sizes. At all other sites the deposition mass remained unchanged (after seasonal variation was taken into account). Since smaller particle sizes have proportionally greater binding sites (Lead et al. 1999), the amount of metal becoming bound with the smaller particles would result in an overall increase in the µg/g ratio. Secondly, the seston metal levels at this site could be reflecting shorter term changes in the effluent metal loads. While over the long term (20 years), metal loads have decreased significantly, Pb and Cu released from the smelter have increased slightly over the shorter term (the three years of the monitoring period [Pasminco Port Pirie, unpub. data]). Alternatively, a lack of transport from the W6 area, a shallow, intertidal region, could be resulting in ever increasing metal loads entering the area and not being exported elsewhere. If this is the case metal concentrations in the area will not change until there is no further discharge from the smelter, and even then the rate of change is likely to be very slow. Since there was no discernible general trend between changes in seston metals, effluent metal loads and metal binding constants for the results at W6, it is thought that a combination of these factors is affecting the overall temporal changes detected in the seston metal levels at W6.

Factors influencing seston metal uptake

The proportion of metal ions that will become bound to particulate matter, and the strength of binding are governed by a number of factors. These include the binding constant of each metal, and the composition of the particulate matter (Balistieri & Murray 1984; Mayer 1989; Decho & Luoma 1994). Additionally, seston characteristics (particle size distribution, total organic carbon) will influence the rate and amount of metal becoming associated with organism tissue. In this study no relationships were found between either TOC content or particle size of seston and metal concentrations in the bivalves, suggesting that the major influence in tissue metal level is the total concentration of the metals in the seston.

Seston metal concentrations and bivalve tissue concentrations

A primary purpose of this study was to assess the relationship between seston metal levels in the Upper Spencer Gulf and uptake of metals by filter-feeding bivalves. Positive relationships between seston and tissue metals were detected with Pb and Zn, although the relationship did not hold in some cases when extreme values were removed. While metals can be taken up through the dissolved fraction or ingestion of particulate form (Luoma 1995), of the five metals in this study, Pb is the one with the highest binding constant for suspended matter (Balistieri & Murray 1984). This suggests that it is likely that the majority of environmental lead will be in particulate form. Extremes of Zn contamination (as defined by elevated levels of Zn in the seston) were reflected in elevated levels of metals in both bivalves. However, this relationship only held with the scallops once the extremes were removed, suggesting *M. edulis* were regulating Zn levels. This is supported by the results from the temporal uptake study. Rainbow (1995) noted that while mussels reflect extreme changes in Zn bioavailability, they do not show lesser changes in accumulated body concentrations of Zn. Scallops are therefore possibly better monitors of the bioavailable fraction of Zn in the environment. Conversely, a relationship between Cd in seston and mussels was detected that was not present with scallops.

No relationships were detected between seston and bivalve tissue Cu (all exposures) and Mn (except scallops in summer). There are a number of possible reasons for this. Firstly, it may be because the particulate metal is not related to the proportion of bioavailable metal (possibly because the bioavailable fraction is mainly the dissolved fraction). Secondly, tissue metal may be being regulated by the organism (either pre- and post-assimilation). Bivalves have been shown to be capable of modifying the digestive processing of food to reduce exposure to high, bioavailable Cr concentrations (Decho & Luoma 1996). Thirdly, individual bivalve variability may have been too great to determine the "true" uptake or the exposure time was too short, and the measured tissue concentrations were not representative of total possible uptake. Additionally, number of other factors (such as the composition of the seston) may differ at each site and therefore affect the relationship between total seston metal and bivalve uptake. For example, metals associated with natural seston are generally assimilated at lower efficiencies than metals bound with unicellular algae (Chong & Wang 2000).

While total cadmium in *P. alba* has been shown to be dependent on the locality of scallop collection (Walker *et al.* 1982), this was not found in this study. Notably, scallops from all sites (including the reference collection site) had levels of Cd above that recommended by ANZFA (2000). The digestive

gland of the scallop *Pecten maximus* Linnaeus has been found to contain high concentrations of Cd, even when collected from apparently unpolluted waters (Rainbow 1997). Rainbow (1997) noted abnormally high tissue Cd concentrations in scallops, and suggested that this is a result of very high feeding rates. Cu uptake in mussels was more closely related to seston Cu levels than in scallops. However, it has been suggested that mussels are not good indicators of environmental concentrations of Cu because they are good regulators of this metal (Rainbow 1995). Allison *et al.* (1998) found that Zn, Cu and Mn in pseudofaeces were often higher than levels in the suspended particulate matter (in laboratory exposures), indicating that the mussel (*M. edulis*) had rejected particles enriched in these metals.

Mussel as a surrogate scallop species

There were significant differences between species in both total tissue metal concentrations and relationship with seston metals. Given the differences between two bivalve species, to assume metals in bivalves represent the bioavailable fraction for all species is erroneous. As Rainbow (1995) notes, a suite of biomonitors, reflecting all metal bioavailability in all available sources is necessary. Using *M. edulis* provides the opportunity to make comparisons between sites, which is very important. However, *P. alba* is an indigenous species, and therefore possibly more relevant to the particular system under investigation.

Conclusions

Long term, frequent monitoring of temporally suspended seston is an appropriate method of assessing trace metal distribution. The only way to elucidate trends in metal levels is to acquire sufficient data to incorporate variation caused by seasonal influences. These data indicate that seston metal concentrations are decreasing at most sites within the Upper Spencer Gulf, reflecting long term overall load decreases from the smelter. Metal levels in seston at the site closest to the smelter have not decreased. This is either a reflection of the shorter term metal loading from the smelter, possibly a result of smaller, metal rich particles being released following ponding of the effluent, or because there is little exchange with the open sea at this site.

Seston Pb is a reasonable indicator of the spatial distribution of Pb among bivalves in the Upper Spencer Gulf. The relationships between Zn, Cd, Cu and Mn seston and the bivalves was variable, although one or both bivalves were able to detect extremes for most metals.

References

ALLISON, N., MILLWARD, G. E. & JONES, M. B. (1998) Particle processing by *Mytilus edulis*: effects on bioavailability of metals. *J. Exp. Mar. Biol. Ecol.* **222**, 149–162.

AUSTRALIA NEW ZEALAND NATIONAL FOOD AUTHORITY (ANZFA) (1996) "Food standards code" (including amendments). (Australian Government Publishing Service, Canberra).

AUSTRALIAN BUREAU OF METEOROLOGY (http://www.bom.gov.au) accessed 22/4/01.

BALISTRIERI, L. S. & MURRAY, J. W. (1984) Marine scavenging: Trace metal adsorption by interfacial sediment from MANOP Site H1. *Geochim. Cosmochim. Ac.* **48**, 921–929.

BOLAND, J. (1995) Time-series analysis of climatic variables. *Solar Energy* **55**, 377–388.

CHONG, K. & WANG, W. X. (2000) Assimilation of cadmium, chromium, and zinc by the green mussel *Perna viridis* and the clam *Ruditapes philippinarum*. *Environ. Toxicol. Chem.* **19**, 1660–1667.

CONNELL, D. W., LAM, P., RICHARDSON, B. & WU, R. (1999) "Introduction to Ecotoxicology". (Oxford, Malden, MA, USA).

DECHO, A. W. & LUOMA, S. N. (1994) Humic and fulvic acids - sink or source in the availability of metals to marine bivalves *Macoma balthia* and *Potamocorbula amurensis*. *Mar. Ecol. Prog. Ser.* **108**, 133–145.

————— & ————— (1996) Flexible digestion strategies and trace metal assimilation in marine bivalves. *Limnol. Oceanogr.* **41**, 568–572.

FERGUSON, J. (1983) Concentrations and speciation of lead, zinc and cadmium in seawater-like smelter effluent and adjacent marine environments, Port Pirie, South Australia. *Aust. J. Mar. Freshw. Res.* **34**, 375–385.

GEORGE, G. S. (1980) Correlation of metal accumulation in mussels with the mechanisms of uptake, metabolism and detoxification: biochemical and ultrastructural studies. *Thalassia jugoslav.* **16**, 347–365.

LEAD, J. R., HAMILTON-TAYLOR, J., DAVISON, W., HARPER, M. (1999) Trace metal sorption by natural particles and coarse colloids. *Geochim. Cosmochim. Ac.* **63**, 1661–1670.

LUOMA, S. N. (1995) Prediction of metal toxicity in nature from bioassays: limitations and research needs. pp 609–660. Tessier, A. & Turner, D.R. (Eds) "Metal Speciation and Bioavailability in Aquatic Systems" (Wiley, Chichester, NY, USA).

MAYER, L. M. (1989) Geochemistry of humic substances in estuarine environments. In: Aiken GR, McKnight DM, Wershaw RL, MacCarthy P (ed). Humic Substances in Soil, Sediment and Water, Wiley, New York, NY, USA.

MORSE, J. W., PRESLEY, B. J., TAYLOR, R. J., BENOIT, G. & SANTSCHI, P. (1993) Trace metal chemistry of Galveston Bay: water, sediments and biota. *Mar. Environ. Res.* **36**, 1–37.

NELSON, D. W. & SOMMERS, L. E. (1982) Total carbon, organic carbon and organic matter. Page. A.L. (Ed). "Methods of soil analysis. Part 2. Chemical and microbiological properties" (American Society of Agronomy, Madison, WI, USA).

NEWMAN, M. C. (1998) "Fundamentals of Ecotoxicology" (Sleeping Bear Press, Chelsea, MI, USA).

PUIG, P., PALANQUES, A., SANCHEZ-CABEZA, J.A. & MASQUÉ, P. (1999) Heavy metals in particulate matter and sediments in the southern Barcelona sedimentation system (North-western Mediterranean). *Mar. Chem.* **63**, 311-329.

RAINBOW, P. S. (1995) Biomonitoring of heavy metal availability in the marine environment. *Mar. Pollut. Bull.* **31**, 183-192.

_____. (1997) Trace metal accumulation in marine invertebrates: marine biology or marine chemistry? *J. Mar. Biol. Ass. U.K* **77**, 195- 210.

REINFELDER, J. R., FISHER, N. S., LUOMA, S. N., NICHOLS, J. W. & WANG, W. X. (1998) Trace metal element trophic transfer in aquatic organisms: a critique of the kinetic model approach. *Sci. Total Environ.* **219**, 117-135.

SMALL, A. C. & HAAS, H. A. (1997) Seston dynamics and food availability on mussels and cockle beds. *Estuar. Coast Shelf Sci*, **45**, 247-259.

TAPPIN, A. D., MILLWARD, G. E., STATHAM, P. J., BURTON, J. D. & MORRIS, A. W. (1995) Trace metals in the Central and Southern North Sea. *Estuar. Coast Shelf Sci*, **41**, 275-323.

TURNER, A. & MILLWARD, G. E. (2000) Particle dynamics and trace metal reactivity in estuarine plumes. *Ibid*, **50**, 761-774.

WALKER, T.I., GLOVER, J. W. & POWELL, D. G. M. (1982) Effects of length, locality and tissue type on mercury and cadmium content of the commercial scallop, *Pecten alba* Tate, from Port Phillip Bay, Victoria. *Aust. J. Mar. Freshwater Res*, **33**, 547-552.

WANG, W. X. & FISHER, N. S. (1996) Assimilation of trace elements ingested by the mussel *Mytilus edulis*: effects of algal food abundance. *Mar. Ecol. Prog. Ser*, **129**, 165-176.

WARD, T. J. & CORRELL, R. L. (1992) Estimating background concentrations of heavy metals in the marine environment. Proceedings of a Bioaccumulation Workshop: Assessment of the Distribution, Impacts and Bioaccumulation of Contaminants in Aquatic Environments. A. G. Miskiewicz, Sydney, Australia, Australian Marine Sciences Association Inc. 133-139.

_____, HUTCHINGS, P. A. (1996) Effects of trace metals on infaunal species composition in polluted intertidal and subtidal marine sediments near a lead smelter, Spencer Gulf, South Australia. *Mar. Ecol. Prog. Series* **135**, 123-135.

_____, WARREN, L. J. & SWAINE, D. J. (1982) "Effects of heavy metal on aquatic life." Final report ILZRO Project CH-6/ZH212, Presented to the International lead zinc research organization by the Commonwealth Scientific and Industrial Research Organisation, December 1982.

_____, WARREN, L. J. & TILLER, K. G. (1984) The distribution and effects of metals in the marine environment near a lead-zinc smelter, South Australia, pp1-76. Nriagu, J. (Ed.) "Environmental Impacts of Smelters". (Wiley, New York, USA).

_____, YOUNG, P. C. (1984) Effects of metals and sediment particle size on the species composition of the epifauna of Pinna bicolor near a lead smelter, Spencer Gulf, South Australia. Estuar Coast Shelf Sci **18**, 79-95.

ZANN, L. P. (1995) "Our Sea, Our Future: State of the Marine Environment Report for Australia"(Great Barrier Reef Marine Park Authority, Queensland, Australia).

ZAR, J. H. (1984) Biostatistical Analysis. Prentice-Hall, Englewood Cliffs, USA.